Towards an Understanding of the Cd Isotope Fractionation during Transfer from the Soil to the Cereal Grain

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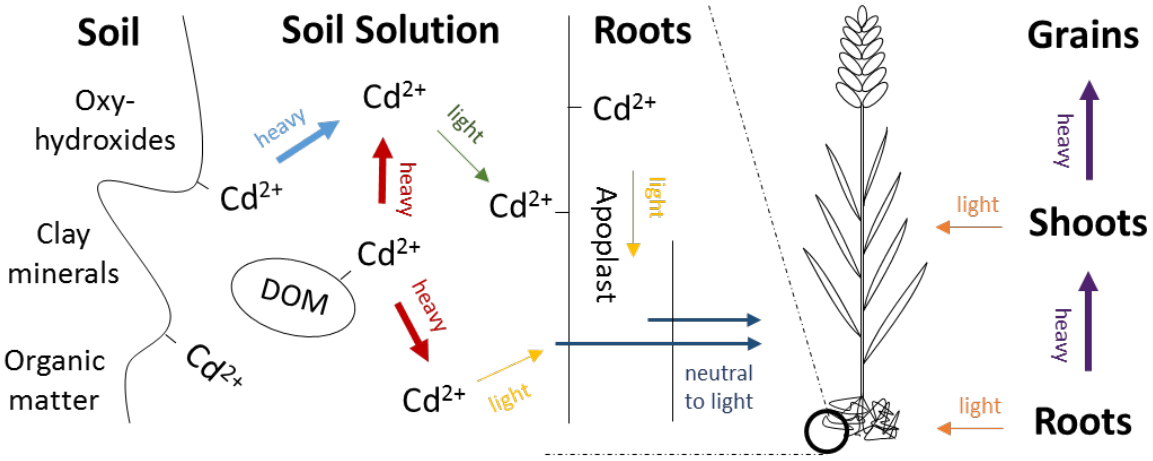
Abstract

Cd in soils might be taken up by plants, enter the food chain and endanger human health. This study investigates the isotopic fractionation of major processes during the Cd transfer from soils to cereal grains. Thereto, soil, soil solution, wheat and barley plants (roots, straw and grains) were sampled in the field at three study sites during two vegetation periods. Cd concentrations and $\delta^{114/110}Cd$ values were determined in all samples, soil solution compositions measured and dissolved Cd speciation modelled. Isotopic fractionation between soils and soil solutions ($\Delta^{114/110}Cd_{20\text{-}50cm\text{-}soil\ solution}$ = -0.61 to -0.68‰) was nearly constant among the three soils. Cd isotope compositions in plants were heavier than in soils ($\Delta^{114/110}Cd_{0\text{-}20cm\text{-}plants}$ = -0.55 to -0.31‰) but lighter than in soil solutions ($\Delta^{114/110}Cd_{soil\ solution\text{-}plants}$ = 0.06 to 0.36‰) and these differences correlated with Cd plant-uptake rates. In a preliminary model, desorption from soil, soil solution speciation, adsorption on root surfaces, diffusion, and plant uptake were identified as the responsible processes for the Cd isotope fractionation between soil, soil solution and plants whereat the first two processes dominated over the last three processes. Within plants, compartments with lower Cd concentrations

were enriched in light isotopes what might be a consequence of Cd retention mechanisms, following a Rayleigh fractionation, in which barley cultivars were more efficient than wheat cultivars.

Capsule: The isotopic fractionation between soil and soil solution is mainly driven by pool-size effects during desorption from the soil solid phase, which also controls Cd isotopes in plants, while the plant internal fractionation is controlled by Cd retention mechanisms which are more efficient in barley compared to wheat plants.

Graphical Abstract



Highlights

- The chemical characteristics of soil and solution does not affect isotope fractionation.
- Desorption and solution speciation control Cd isotopic composition of the plants.
- Cd retention controls the plant internal Cd isotope distribution in the plant.
- Barley plants are more effective in preventing Cd to be transferred to the grain.

Keywords: Cadmium, cereal, plant metal uptake, soil, soil solution

Introduction

Cadmium is a toxic element of major concern, which accumulates in many agricultural soils because of deposition from the atmosphere, mineral phosphate (P) fertilization, and the amendment of various materials, including compost, sewage sludge, waste water, and industrial by-products (Alloway and Steinnes, 1999). Within soils, Cd can desorb from solids into dissolved form, be taken up by crops, and thus enter the human food chain (Nagajyoti et al., 2010). Such uptake is undesired, as Cd is toxic for plants and humans and accumulates in human bodies (Godt et al., 2006). Even low Cd concentrations in edible plant parts can pose a risk for human health because the biological half-life of Cd is 10-30 years (Nordberg, 2009). To assure low Cd concentrations in crops, it is therefore important to better understand plant-uptake and plant-internal redistribution processes.

The path of Cd from soils to plant tissues involves the three steps of desorption, plant-uptake, and plant-internal redistribution. Each of these steps might involve isotopic fractionation. First, most Cd in soils is stored in the solid phase in various chemical forms (Wilcke et al., 2005) and only a minor part dissolves in the soil solution (Bruemmer et al., 1986) mainly determined by soil pH (Bruemmer et al., 1986; Christensen, 1984; Herms and Brümmer, 1984; Sauvé et al., 2000). In soil solutions, Cd is either present as inorganic free Cd^{2+} or forms a number of complexes (Nolan et al., 2003). Plants can alter their rhizosphere soils with the excretion of a wide range of organic (e.g., acids) and inorganic (e.g., H^{+}) substances (Clemens et al., 2002; Welch, 1995). Consequently, the share of the plant available Cd might increase (Awad and Römheld, 2000; Grayston et al., 1997; Treeby et al., 1989; Dakora and Phillips, 2002; Cancès et al., 2003). Second, non-specific uptake of Cd has been revealed for plants – with metal transporters that take up essential trace metals (Clemens et al., 2002; Perfus-Barbeoch et al., 2002; Clemens and Ma, 2016; Mendoza-Cózatl et al., 2011). Because rhizosphere pH can be altered by plants, soil Cd concentrations appear to mainly determine crop Cd concentrations (Clemens et al., 2002; Dakora and Phillips, 2002). Third, living cells developed strategies to avoid cytosolic Cd^{2+} and prevent toxic effects; these strategies influence Cd concentrations in plant tissues and may be similar for bacteria and human cells (Prévéral et al., 2009). For Cd concentrations in grains of wheat and barley, xylem and phloem transport are important (Kubo et al., 2016; Chen et al., 2007).

77 To investigate Cd behavior in soils and its uptake into plants Cd stable isotopes might offer
78 important information about biogeochemical processes. Cd in solution has been shown to be
79 isotopically heavier than Cd in solid phases in leaching, sorption and coprecipitation processes
80 ($\Delta^{114/110}Cd_{solid\text{-}fluid}$ = -0.54 to -0.24‰, Horner et al., 2011; Wasylenki et al., 2014; Zhang et al.,
81 2016). Living cells were enriched in light isotopes probably through Cd sequestration into cell
82 walls (Horner et al., 2013; McBride, 2002) and Cd-tolerant plants showed the same pattern
83 ($\Delta^{114/110}Cd_{solution\ -\ plant}$ = 0.30 to 0.46‰, Wei et al., 2016). In an earlier study of our group, pot
84 experiments were done on two of the soils used here. Wheat plants were shown to be
85 depleted in Cd and to get isotopically heavier from roots to straw and from straw to grains
86 (Wiggenhauser et al., 2016). These fractionations were explained by Cd binding to S-ligands
87 such as glutathione and phytochelatin; possibly with subsequent excretion or sequestration
88 into vacuoles (Song et al. 2014; Dorcak and Krezel, 2003; Maret and Moulis, 2013; Mendoza-
89 Cózatl et al., 2011; Prévéral et al., 2009). As a consequence, light isotopes were preferentially
90 sequestered, which is also predicted by theoretical calculations (Wiggenhauser et al., 2016;
91 Yang et al., 2015).

92 We formerly established Cd balances and calculated Cd stable isotopes fluxes at the three
93 sites which are also presented in this paper (Imseng et al. 2018). Thereby, the anthropogenic
94 impact on Cd concentrations in the soils could be assessed and important natural long-term
95 processes were identified (Imseng et al. 2018). However, to understand Cd behavior and
96 isotope fractionation in the soil-plant system, further research on Cd partitioning and Cd
97 isotope fractionation between soil, soil solution and plants is necessary. Finally, by now, no
98 field data about Cd isotope fractionation in wheat are available and data from other cereals
99 are generally missing. To fill this research gap, Cd concentrations and Cd isotope compositions
100 of soil, soil solutions and three plant compartments of wheat and barley were determined
101 and Cd speciation in soil solution modelled. This knowledge will be important to further
102 establish stable isotope tools for the investigation of Cd uptake into plants and thus to avoid
103 Cd uptake into crops and the human food chain. Wheat was chosen because it is the most
104 important food crop in Europe; barley as well is of economic importance and is often
105 cultivated in crop rotation on the same soils as wheat (Leff et al. 2004). In this study, the
106 following aims were aspired: (i) understand the influence of soil properties and Cd speciation
107 in the soil solution on isotopic fractionation between soil and soil solution, (ii) assess the

relative importance of the five processes (#1) desorption from soil, (#2) soil solution speciation, (#3) adsorption on root surfaces, (#4) diffusion, and (#5) plant uptake for the overall isotope composition differences between soils and plants, and (iii) investigate the plant internal Cd redistribution in wheat and barley plants.

Materials and Methods

Study Sites and Sampling

The three study sites Oensingen (OE), Wiedlisbach (WI), and Nenzlingen (NE, Figure S1) as well as the sampling of soils and soil solutions were already described in a recent study (Imseng et al., 2018). Entire plants were harvested at full maturity, in summers 2014 and 2015. The entire bulk root system was thereby sampled by removing a 20 cm-deep soil monolith with a surface area of 400 cm^2. During the first season, winter wheat was grown at OE (*Triticum aestivum L. cv. Zinal*) and NE (*Triticum aestivum L. cv. Mulan*) and summer wheat at WI (*Triticum aestivum L. cv. Fiorina*). During the second season, winter barley was grown at OE (*Hordeum vulgare L. cv. Meridian*), WI (*Hordeum vulgare L. cv. Classic*) and NE (*Hordeum vulgare L. cv. Caravan*).

Laboratory Analysis

From all soil horizons, 0.05 M $Ca(NO_3)_2$ extractable Cd was determined (Gray et al., 1999), followed by hot plate digestion with 40% HNO_3 to oxidize extracted organic complexes. Cadmium concentrations were measured with inductively-coupled plasma mass spectrometry (ICP-MS, 7700x, Agilent Technology, Waldbronn, Germany). Plants were separated into grains, straw (stem and leaves) and roots (Wiggenhauser et al., 2016). Grains and straw were dried at 60 °C for 48 h. Roots were rinsed with 6 mM $NaNO_3$ until no visible rhizosphere soil was left (Wiggenhauser et al., 2016) and dried at 60 °C for 48 h. The dried grains, straw and roots were weighed for the determination of the dry matter distribution, cut with a knife mill (GM 200, Retsch, Haan, Germany) and further ground with a planetary ball mill with agate beakers (PM 200, Retsch, Haan, Germany). Immediately after sampling of soil solutions, pH was determined in an aliquot that was afterwards discarded. For the soil solutions, the concentrations of major cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) and anions (Cl^-, NO_2^-, NO_3^-, PO_4^{2-} and SO_4^{2-}) were measured by ion chromatography (IC, DX-120, ThermoFisher Scientific, Waltham, MA, USA) after filtration with 0.45 µm syringe filters (Whatman PVDF). The dissolved organic carbon (DOC) concentrations were analyzed with a vario TOC cube analyzer (Elementar Analysensysteme, Langenselbold, Germany) and total element concentrations (Al,

V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Ba, La, Ce, Pb, Th and U) were analyzed by ICP-MS.

Stable Isotope Measurements

For the isotopic measurements, all samples were prepared in order to yield ≥100 ng of Cd for isotopic analysis (Wiggenhauser et al., 2016). As soil solution samples from OE and NE had very low Cd concentrations, between 1.1 and 5.3 L of soil solution were combined to acquire at least 10 ng Cd for isotopic analysis and evaporated prior to digestion. Because of the low plant Cd concentrations, wheat and barley samples were pre-digested in a microwave oven (ETHOS, MLS, Leutkirch, Germany). Depending on the Cd concentration, 0.5-3.0 g of sample were digested in 10 mL HNO_3 (69%) at 200°C for 0.5 h, transferred to Savillex® beakers (Savillex Corporation, Eden Prairie, MN, USA) and evaporated to dryness on a hot plate (120 °C). Evaporated water samples, pre-digested plant samples and between 0.1 to 0.3 g of soil samples were digested on the hot plate (120 °C, 48 h) in 3 mL HNO_3 (69%), 1 mL H_2O_2 (30%) and 2 mL HF (40%). The digests were evaporated to dryness, re-dissolved 4 mL aqua regia (120 °C, 48 h) and dried again. The samples were then dissolved in 5 M HCl and Cd concentrations were determined on small solution aliquots by ICP-MS. A $^{111}Cd/^{113}Cd$ double spike solution was added to the samples to obtain a ratio of spike-to sample-derived Cd of ~1.2 and equilibrated for 24 h. Cadmium was then separated from the sample matrix using a three-stage column chemistry that employs both anion exchange and extraction chromatography (Murphy et al., 2016; Xue et al., 2012) This was followed by a liquid-liquid extraction step for further sample cleanup (Murphy et al., 2016).

The Cd isotope compositions were then determined on a Nu-Plasma HR multiple collector inductively-coupled plasma mass spectrometer (MC-ICP-MS, Nu Instruments Ltd, Wrexham, UK) at the Imperial College London MAGIC Laboratories. The isotope compositions of all samples were measured using standard-sample bracketing and using the added double-spike for correction of instrumental mass bias (Murphy et al., 2016; Xue et al., 2012). The total procedural Cd blank (n=11) for the isotopic measurements ranged from 110 to 1011 pg. This is equivalent to less than 2.5% of the smallest indigenous Cd mass among the samples, whilst the typical blank proportion was about 0.4%. Hence, no blank corrections were required for

171 the isotopic data. Several standard reference materials (SRMs) were analyzed together with
172 the samples for quality control and the results show good agreement with previously
173 published values (Table S3). The double-spike method also yields precise Cd concentrations
174 (Ripperger and Rehkämper, 2007). For the SRMs, the measured Cd concentrations were
175 slightly lower than the certified values but our data are in in line with the results of other
176 recent studies (Goix et al., 2011; Jochum et al., 2005; Wiseman et al., 2013).

177

178 *Modelling and Calculations*

179 Cd speciation in the soil solution was calculated with Visual MINTEQ (Table S1). The pH,
180 cation, anion, total element and dissolved organic matter concentration from the analyzed
181 soil solution samples were used as input data. Ionic strength was calculated based on the ion
182 and element concentrations, by calculating the sum of all positive charges in solution (cation
183 concentration x cation charge). Dissolved organic matter (DOM) concentrations were inferred
184 from the measured dissolved organic carbon concentrations (DOC), using a converting factor
185 DOM:DOC of 2:1 (Pribyl, 2010). Cadmium speciation was modelled with two scenarios,
186 because it has been reported that the DOM composition is an important source of
187 uncertainty (Groenenberg et al., 2010). In the min-HS and max-HS scenario, DOM consisted to
188 20% and 60% of humic hydrophobic substances (HS), respectively, in line with reported values
189 (Groenenberg et al., 2010; Ren et al., 2015). The remaining mass fractions were assumed to
190 consist of hydrophilic organic substances. This fraction can be neglected in the models
191 because it was shown that they do not influence Cd speciation (Groenenberg et al., 2010; Ren
192 et al., 2015). In both scenarios, humic substances were subdivided into fulvic (FA, 90%) and
193 humic (HA, 10%) acids (Groenenberg et al., 2010; Ren et al., 2015). DOM in the min-HS
194 scenario therefore consists to 2% of humic acids, 18% of fulvic acids and 80% of hydrophilic
195 organic substances whilst in the max-HS scenario it is comprised of 6% humic acids, 54% fulvic
196 acids and 40% hydrophilic substances.

197 To calculate the expected Cd stock in plants, the crop transpiration (T_c) was calculated by
198 correcting the reference evapotranspiration ET_0 with the basal crop coefficient (K_{cb}, Allen,
199 1998). To this end, ET_0 was calculated according the FAO Penman-Monteith method (Allen,
200 1998) whilst K_{cb} was obtained from the meteorological (wind speed and relative humidity)

and agricultural (crop type, growth stage and crop heights) data provided by the Federal Office of Meteorology and Climatology (MeteoSwiss, 2015) and derived from the biweekly observations at the study sites, respectively. For the meteorological data, the stations in Wynau (for OE and WI) and Basel/Binningen (for NE) were chosen (Figure S1, MeteoSwiss, 2015).

The expected Cd stock of plants was determined by multiplying the biweekly measured soil solution Cd concentration with the biweekly data for the calculated crop transpiration of wheat per area. The concept of the expected Cd stock in plants assumes that the plants do not alter their rhizosphere soil and take up the soil solution with its measured Cd concentration. The actual Cd stock in wheat and barley plants was calculated from the measured Cd concentration in plant parts (root, straw and grains) and the farmers' data on the dry matter of harvested straw and grains per area. The dry matter of roots per area was derived from the contribution of root-associated dry matter to that of the harvested plants. Because wheat and barley plants were only harvested once per site, standard deviations could not be calculated for the dry matter distribution. Hence, standard deviations for dry matter were assumed to be the same as in a previous pot study, partly conducted on the same soils (Wiggenhauser et al., 2016).

The Cd isotope compositions of the samples are reported relative to the NIST 3108 Cd isotope reference material using a δ notation based on the $^{114}Cd/^{110}Cd$ ratio (Equation 1).

$$Equation\ 1: \quad \delta^{114/110}Cd = \left[\frac{(^{114}Cd/^{110}Cd)_{sample}}{(^{114}Cd/^{110}Cd)_{NIST3108}} - 1\right] \cdot 1000$$

The $\Delta^{114/110}Cd$ values, which denote the apparent isotopic fractionation between two sample types (e.g., between soil and soil solution) were calculated according to Equation 2 with an error propagation according to Kusonwiriyawong et al. (2017).

$$Equation\ 2: \quad \Delta^{114/110}Cd_{soil-soilsolution} = \delta^{114/110}Cd_{soil} - \delta^{114/110}Cd_{soilsolution}$$

The isotopic compositions of whole plants or whole-year soil solutions are comprised of several fractions (e.g., wheat plant = roots + straw + grains). The average isotopic composition

was calculated according to Equation 3 using an error propagation according to Kusonwiriyawong et al. (2017).

$$Equation\ 3: \quad \delta^{114/110}Cd = \frac{\sum_{f=1}^{n} \delta^{114/110}Cd_f \cdot mCd_f}{\sum_{f=1}^{n} mCd_f}$$

$\delta^{114/110}Cd$: isotopic ratio of the whole plant
$\delta^{114/110}Cd_f$: isotopic ratio of a plant compartment
mCd_f: Cadmium mass of a compartment

The variability of the isotope compositions between the different plants was calculated by comparing the $\Delta^{114/110}Cd_{grains\text{-}roots}$ for each site and plant. The statistical significance was subsequently calculated using ANOVA with a level of significance of $p < 0.05$.

Results

At all three sites, the extractable soil Cd concentrations decreased with depth (Figure S2a). On average, they were highest at WI (18.1 µg kg^{-1}), followed by NE (5.2 µg kg^{-1}) and OE (4.3 µg kg^{-1}). All other considered soil properties including total soil Cd concentrations and pH were published in a recent study (Imseng et al., 2018) and are shown in Table S2. Also the bulk plant and soil solution Cd isotope analyses are already reported there. So in this study we focus on a higher temporal variation, different plant compartments and a more detailed chemical characterization of the soil solution. The averaged pH of the biweekly sampled soil solutions was lowest at WI (6.0) and higher at OE (7.8) and NE (7.6, Figure 1, Table S1). In line with soil pH, the average soil solution Cd concentration at WI (156 ng L^{-1}) exceeded the values at NE (11.3 ng L^{-1}) and OE (3.2 ng L^{-1}), by one or two orders of magnitude, respectively. In contrast to pH and dissolved Cd concentrations, the DOC concentrations (2.6 to 5.6 mg L^{-1}) and ionic strengths (1.7 to 11.8 mmol L^{-1}) displayed only limited variability among the three sites. Modelling of Cd speciation revealed that dissolved Cd was either bound to DOM or present as free Cd^{2+} whilst other species contributed less than 5% to the dissolved Cd concentration (Table S1). On average, the share of free Cd^{2+} was higher in the min-HS than in the max-HS scenario at OE (38 and 13%), WI (70 and 41%) and NE (49 and 20%). Furthermore, the proportion of free Cd^{2+} to the total Cd concentrations typically increased with decreasing pH (Figure 1).

The Cd concentrations of wheat and barley plants at all sites decreased from roots (208-465 ng g^{-1}) to straw (65-247 ng g^{-1}) to grains (17-98 ng g^{-1}, Figures S3a & S3b). Among the sites, the Cd concentrations of the whole wheat and barley plants correlated with the Cd concentrations of the solid soils (Figure S4), but not of the soil solution. The Cd concentrations of individual wheat and barley plant parts correlated with the soil Cd concentrations (Figure S4), except for barley roots, where the concentrations at WI (221 ng g^{-1}) were higher than at OE (431 ng g^{-1}). Straw contributed most to the dry matter (54 to 60%) of wheat plants at all three sites (Figures S3c and S3d) whilst grains provided most of the dry matter for barley plants (56 to 60%). For both plants and at all sites, the roots contributed the smallest proportion to dry matter. Moreover, the dry matter distribution among the plant parts of both crops was similar for the three sites, despite growing of different wheat and barley cultivars at the three sites, respectively. For both plants, most of the Cd was stored in

271 straw (54 to 80%, Figures S3e and S3f), followed by grains (15 to 28%) and roots (5 to 24%).
272 An exception to this are the barley roots from WI as these contained more Cd (at 24%) than
273 barley grains (21%). Overall, Cd was distributed more uniformly in barley than in wheat, as the
274 latter displays larger differences in Cd content between roots, straw and grains. Crop
275 transpiration (T_c) was calculated for the complete wheat-cultivation period and similar values
276 of 415, 530, and 507 L m^{-2} were determined for OE, WI and NE, respectively. The expected Cd
277 stock in wheat was $0.07x10^4$, $3.54x10^4$, and $0.24x10^4$ ng m^{-2} at OE, WI and NE, respectively. In
278 contrast, the measured Cd stock was about two orders of magnitude higher both at OE
279 ($15.70x10^4$ and $3.85x10^4$ ng m^{-2} for wheat and barley, respectively) and NE ($18.95x10^4$ and
280 $9.05x10^4$ ng m^{-2}) but close to the expected value at WI ($5.94x10^4$ and $4.85x10^4$ ng m^{-2}).

281 The isotopic compositions of bulk soils (Imseng et al., 2018), soil solutions (Imseng et al.,
282 2018), and the $Ca(NO_3)_2$ extracts of soils (Wiggenhauser et al., 2016) were published in recent
283 articles. Compared to the soils, the whole wheat and barley plants were enriched in heavy
284 isotopes and the isotopic fractionation between the soil and whole plants showed only
285 limited variability between the sites for both wheat ($\Delta^{114/110}Cd_{soil(0\text{-}20cm)\text{-}wheat}$ = -0.31 to -
286 0.46‰) and barley ($\Delta^{114/110}Cd_{soil(0\text{-}20cm)\text{-}barley}$ = -0.51 to -0.55‰, Figure 2a). A comparison of the
287 isotopic data for soil solutions and whole plants thereby indicates that light Cd isotopes are
288 more strongly enriched in wheat ($\Delta^{114/110}Cd_{soil\ solution\text{-}wheat}$ = 0.20 to 0.36‰) and barley
289 ($\Delta^{114/110}Cd_{soil\ solution\text{-}barley}$ = 0.06 to 0.18‰) than in the soil solutions. Within wheat and barley
290 plants of all three sites, the $\delta^{114/110}Cd$ values increased systematically from roots (-0.05 to
291 0.20‰, Figure 2b) to straw (0.09 to 0.51‰) and grains (0.47 to 1.32‰). The Cd isotope
292 compositions of the plant parts were thereby significantly more variable in barley
293 ($\delta^{114/110}Cd_{barley\ plant\ parts}$ = 0.06 to 1.32‰) than in wheat ($\delta^{114/110}Cd_{wheat\ plant\ parts}$ = -0.05 to
294 0.77‰), whilst Cd was more uniformly distributed in the former.

295

Discussion

Cd in Soil and Soil Solution

The pH of soils and soil solutions mainly controlled the extractable Cd, dissolved Cd in soil solutions, and the share of free Cd^{2+} in the same solutions and thus the plant available Cd. The DOM concentration and composition had a minor influence on the plant available Cd. First, a comparison of the pH as well as the total and extractable Cd concentration data for the three sites at each depth reveals that the extractable Cd, similar to the Cd concentrations in soil solutions, was mainly influenced by soil pH and not by total Cd concentrations (Figure S2). The extractable Cd concentrations decreased with depth at all three sites. In the upper soil horizons, Cd may originate from decomposed organic matter, mineral fertilizers and manure, while in deeper soil horizons, Cd may be increasingly derived from unweathered minerals (Schweizer et al. 2018; Loganathan and Hedley, 1996; Degryse e al., 2003; de Groot et al., 1998). Second, the soil solution pH not only correlated negatively with soil solution Cd concentrations (Figure 1a, Table S1), but also affected the Cd speciation in soil solutions with a trend to higher proportions of free (plant available) Cd^{2+} at lower pH values (Figures 1b and 1c, Table S1). WI was the site with highest fractions of free Cd^{2+} probably because the soil has the lowest pH and the highest dissolved Cd concentrations. For OE and NE, additional calculations with Visual MINTEQ (data not shown) revealed that the share of free Cd^{2+} should increase with both lower pH and higher dissolved Cd concentrations, when all other solution properties are kept constant. Finally, the DOM content and composition impacts the Cd speciation. In the min-HS scenario (20% of DOM are humic substances), more of the soil solution Cd is present as free Cd^{2+} than in the max-HS scenario (60% humic substances). This is reasonable because humic substances, which consist mainly of fulvic acids, are the main cation sorbent in soil solutions (Groenenberg et al., 2010; Ren et al., 2015). Hence, increasing the DOM concentrations at a constant contribution of humic substances to DOM will decrease the share of free Cd^{2+}.

The average isotopic fractionation between the soil solutions and the soils varied little among the sites with a consistent enrichment of the heavy isotopes in the former ($\Delta^{114/110}Cd_{soil(20\text{-}50cm)\text{-}soil\ solution}$ = -0.61 to -0.68‰, Imseng et al., 2018). The fractionation was thereby not affected by any other property of the soil solid phase (Cd concentration, pH, cation exchange

capacity (CEC), organic matter concentration, texture, Imseng et al., 2018) or soil solution (Cd concentration, pH, Cd speciation, DOM concentration, Table S1). Our data are in line with the results of comparable studies, which showed an enrichment of heavy isotopes in the solution phase, after simulated and natural weathering (Zhang et al., 2016), adsorption to Mn oxyhydroxides (Wasylenki et al., 2014) and co-precipitation with calcite (Horner et al., 2011).

The isotope fractionation pattern of Cd between solid and solution phases can be explained by bond lengths between Cd and its surrounding atoms (Wasylenki et al. 2014). Equilibrium isotope fractionation is associated with an enrichment of the heavier isotopes in the phase with the shorter and stiffer bonds (Wiederhold, 2015). EXAFS studies have revealed that distances between Cd and the neighboring oxygen atoms are slightly shorter for aqueous Cd (2.27 to 2.28 Å) than for Cd carbonates (2.28 Å), Cd bound to goethite or humic acids (2.28 to 2.30 Å), $Cd(NO_3)_2$ (2.30 Å), Cd bound to γ-manganite or cellulose (2.31 Å) and Cd bound to pectin or malate (2.32 Å) (Ohtaki and Johansson, 1981; Vasconcelos et al., 2008; Fulda et al., 2013; Bochatay et al., 2000; Isaure et al., 2015; Karlsson et al., 2005). Even longer distances have been reported for bonds between Cd and sulfur atoms of organic matter (2.51 to 2.56 Å, Fulda et al., 2013; Isaure et al., 2015; Karlsson et al., 2005). These findings explain why several studies consistently reported enrichments of heavy isotopes in solutions relative to various solid phases (Horner et al., 2011; Imseng et al., 2018; Wasylenki et al., 2014; Zhang et al., 2016). Only a few EXAFS studies found Cd-O bonds of adsorbed Cd to be shorter than those of aqueous Cd, namely Cd bound to mineral interlayer or surface oxides (2.16 to 2.24 Å), Cd-glutathione complexes (2.26 Å), Cd bound to kaolinite (2.26 to 2.29 Å) and Cd bound to cysteine or clay minerals (2.27 Å). The enrichment of light isotopes indicates that these sorbents played a minor role for the Cd adsorption in our soils (Malferrari et al., 2007; Isaure et al., 2015; Vasconcelos et al., 2008; Sajidu et al. 2008; Fulda et al., 2013).

In contrast, bonds between Zn (2.06 to 2.11 Å) or Cu (four 1.97 Å and two 2.38 Å) and O for octahedral aqueous species are consistently longer than those for other octahedrally/tetrahedrally complexed Zn (1.94 to 2.06 Å) and Cu (1.85 to 2.12) species (Fulton et al., 2000; Kuzmin et al., 1997; Waychunas et al. 2002; Juillot et al., 2008; Nelson et al., 2017; Peacock and Sherman, 2004). Consequently, Zn and Cu adsorbed to humic substances, silicates, oxyhydroxides, and calcite have been reported as isotopically heavier than the aqueous Cu and Zn species (Bigalke et al., 2010; Jouvin et al., 2009; Ryan et al., 2014; Nelson

et al., 2017; Balistrieri et al., 2008; Bryan et al., 2015; Juillot et al., 2008; Pokrovsky et al., 2008; Dong and Wasylenki, 2016). The reverse bond length pattern between aqueous and adsorbed species for Zn and Cu in comparison to Cd are also reflected in the solution-solid phase partitioning coefficients (K_d values) for humic acids and Fe-oxides which indicate an increasing cation selectivity of the solid phases in the order Ca << Cd < Zn << Cu (Smolders and Mertens, 2013). Accordingly, we would expect that aqueous Ca, like Cd, is also isotopically heavier than the adsorbed Ca and this has been indeed shown in former studies (Hindshaw et al., 2013; Ockert et al., 2013).

The similar direction and extent of isotope fractionation between soil and soil solution for the three sites suggests that the Cd sorption mechanism was similar among the three soils. It is presumably dominated by inner-sphere adsorption to organic matter and hydroxides and not by outer-sphere adsorption to clay minerals. It was reported, that clay minerals are by a factor of 5 more selective for Cd^{2+} than for Ca^{2+} (Farrah et al. 1980). However, the soil solution Cd concentrations were 5 (WI), 7 (NE) and 8 (OE) orders of magnitude below the soil solution Ca concentrations. Therefore, outer-sphere adsorption on clay minerals presumably played an insignificant role for the Cd adsorption in our soils. This is further confirmed by the fact that isotopic fractionation did not differ between the three sites despite CEC_{eff} and pH were higher at OE and NE than at WI (Imseng et al., 2018). Hence, Cd was most probably bound to organic matter and hydroxides as inner-sphere complex (Smolders and Mertens, 2013). Adsorption to organic matter, was reported to dominate in soils with pH <6.5 (i.e. WI) whereas adsorption to Fe-oxides becomes more important at pH >6.5 (i.e. OE and NE, Buekers et al., 2008). Hence, organic matter and Fe-oxides seem to be similar in their Cd isotope fractionation.

The isotope compositions of the $Ca(NO_3)_2$ extractable Cd, determined in a pot study with the same soils of OE and WI (Wiggenhauser et al., 2016), pointed out that a higher ionic strength enhanced the Cd desorption and caused an isotopic pool-size effect by shifting the Cd isotope compositions in the dissolved fraction towards the Cd isotope compositions of the soils. Similar to the soil solution, the $Ca(NO_3)_2$ extractable Cd was isotopically heavier than the Cd of the solid soils ($\Delta^{114/110}Cd_{soil(0\text{-}20cm)\text{-}Ca(NO_3)_2\ extract}$ = -0.44 and -0.31‰). However, the enrichment of heavy isotopes in the extracted Cd was less pronounced than in the Cd from soil solutions (Figure 2). It was shown for Zn that higher ionic strength is associated with a more

388 pronounced enrichment of heavy isotopes on surfaces, most probably because the competing
389 ions occupied the outer-sphere adsorption sites such that Zn adsorbed more specifically as
390 inner-sphere complex with shorter and stiffer bonds (Bryan et al., 2015; Nelson et al., 2017).
391 The ionic strength of our extracts (~0.1 M) was about one order of magnitude higher
392 compared to the soil solutions (~0.01 M). Hence, the Ca^{2+} ions of the 0.05 M $Ca(NO_3)_2$
393 extraction solutions most likely competed with Cd for the inner-sphere sorption sites of
394 organic matter and hydroxides. This is reasonable, given that the K_d values for Ca are only 2 to
395 4 orders of magnitude smaller than those of Cd but the Ca concentrations of the extraction
396 solutions exceeded those of Cd in the soil solutions by 8 to 10 orders of magnitude. This
397 suggests that the Ca ions of the extraction solutions induced desorption of additional inner-
398 spherical bound Cd from organic matter and hydroxides, and led to a pool-size effect
399 (Wiederhold, 2015) by shifting the isotopic composition of the dissolved Cd towards the
400 isotopic composition of the soil Cd. This effect was also reflected in the higher Cd
401 concentrations in the extracts compared to the natural soil solution (Table S1 and S2).

402

403 *Cd Uptake by Plants*

404 Plants acidify rhizosphere soils to mobilize nutrients (Clemens et al., 2002; Welch, 1995) and
405 there is evidence that the wheat and barley plants studied here did accordingly with the side
406 effect of increasing Cd availability. First, Cd concentrations of wheat and barley were related
407 to the Cd concentrations of the soils (Figure S4) rather than the Cd concentrations of the soil
408 solutions. This suggests that the wheat and barley plants created microenvironments in the
409 rhizosphere soil where pH values were lower and more homogeneous among the sites than
410 they were for the bulk soils. As a consequence, the dissolved Cd concentrations of these
411 microenvironments were mainly determined by the bulk soil Cd concentrations (Christensen,
412 1984). Second, the measured Cd stock of the wheat and barley biomass from OE and NE was
413 two orders of magnitude higher than the expected value (Figure S5). A different result was
414 obtained for WI, the site with the lowest pH value, where the measured Cd stock of wheat
415 and barley was similar to the expected value. The large offset observed at OE and NE might
416 be due to the alteration of the rhizosphere soils by the wheat and barley plants and enhanced
417 Cd availability, for example by soil pH reduction through release of organic acids and protons

418 (Collins et al., 2003; Naidu and Harter, 1998). Notably, Visual MINTEQ calculations revealed
419 that both, decreasing solution pH and increasing solution Cd concentrations, enhance the
420 share of free Cd^{2+} in soil solutions. As free Cd^{2+} is generally thought to be the main Cd form
421 that is taken up by plants (Mendoza-Cózatl et al., 2011), the acidification of the rhizosphere
422 might have increased Cd^{2+} uptake by plants. The latter is facilitated by membrane proteins in
423 phytoplankton and plants which transport nutrients as divalent cations, such as Fe^{2+}, Zn^{2+} and
424 Mn^{2+} (Horner et al., 2013; Page and Feller, 2015; Yamaji and Ma, 2014). Because the targeted
425 nutrients are chemically similar to Cd, plants also take up the non-essential Cd (Khan et al.,
426 2014). In contrast, the exudation of phytosiderophores by wheat plants grown on metal-
427 enriched soils, have not been observed to mobilize Cd unlike Cu, Ni and Zn (Puschenreiter et
428 al., 2017).

429 The subsequent preliminary model (Figure 3) describes five processes which together can
430 explain the Cd isotope compositions of whole wheat and barley plants.

431 (#1) *Cd desorption from soils* - desorbed Cd is isotopically heavier than adsorbed Cd: The
432 differences in Cd isotopic composition between solid soils and whole wheat and
433 barley plants could be caused by the isotopic fractionation between the solid and
434 solution phases and the amount of desorbed Cd. Mobilization of Cd is furthermore
435 passively enhanced by plants. Consequently, the isotopic signature of the dissolved
436 Cd, that is taken up by plants, shifts towards Cd isotope compositions of the soils
437 and is lighter than the undisturbed soil solutions but still heavier than the bulk soils.
438 This pool-size effect is also revealed by the relationship between the plant Cd
439 stocks and $\Delta^{114/110}Cd_{soil\text{-}plant}$ values (Figure 4). At OE and NE, the wheat plants
440 contained four and two times more Cd than barley plants, respectively. This might
441 reflect that wheat plants are more efficient in mobilizing Cd than barley plants and
442 hence the former are isotopically lighter than the latter because of the stronger
443 pool-size effect. This phenomenon could be attributed to the same pool-size effect
444 as observed with the Cd extracts and the soil solution, where the smaller mobilized
445 fraction (soil solution and barley) showed the heavier values, while mobilization of
446 more Cd (extracts and wheat) caused the isotope ratios to become lighter. At WI in
447 contrast, wheat and barley plants have about the same Cd stock, in accord with the
448 observation that both plants have similar isotopic compositions.

(#2) *Cd speciation in soil solutions* - free Cd^{2+} is isotopically heavier than Cd associated with DOM: The isotope fractionation between different dissolved Cd species is hitherto unstudied. However, Cd bound to soil surfaces is known to be isotopically lighter than dissolved Cd (Figure 2, Wasylenki et al., 2014; Zhang et al., 2016) and organic matter is considered to be the most important Cd sorbent in soils. Thus, from that theoretical point of view, we would expect the free Cd^{2+} – which is the main form taken up by plants – to be isotopically heavier than DOM-bound Cd (Clemens and Ma, 2016; Perfus-Barbeoch et al., 2002). This expectation is in line with the shorter and stiffer bonds of hydrated Cd (Ohtaki and Johansson, 1981; Vasconcelos et al., 2008) than of Cd bound to the S ligands of organic matter (Karlsson et al., 2005). At our sites, the Cd speciation differed between the sites, with the predominant part of the dissolved Cd being present as free Cd^{2+} at WI and as DOM bound Cd at OE and WI. Despite these differences, the isotopic fractionation between the soil and soil solution was constant among the sites. This is a hint that Cd speciation in solution did not influence the Cd desorption from the soil.

(#3) *Cd adsorption on root surfaces* - adsorbed Cd is isotopically lighter than dissolved Cd: It is well known that the negatively charged root surfaces and the apoplast of plants grown in soils or hydroponics are important sorption sites for Cd^{2+} (Sattelmacher, 2001; Uraguchi et al., 2009). When the mechanisms described above come into play, the adsorbed Cd will be isotopically lighter than the soil solution Cd (Horner et al., 2011; Wasylenki et al., 2014; Zhang et al., 2016).

(#4) *Cd diffusion* - faster movement of light isotopes: It was previously demonstrated that free ions and lighter isotopes diffuse faster than complexed ions and heavier isotopes (Rodushkin et al., 2004). Diffusion therefore supports the preferential movement of light Cd isotopes from soil solutions to root surfaces and from the apoplast solution to metal transporters (Jouvin et al., 2012).

(#5) *Plant uptake of Cd* - with either no isotope fractionation or a slight enrichment of light Cd isotopes: To enter the plant, Cd^{2+} ions generally need to cross a plasma membrane via ion channels or transporters for essential nutrient metals (Clemens and Ma, 2016; Perfus-Barbeoch et al., 2002). Nonspecific Cd uptake by

phytoplankton is not associated with isotopic fractionation because of fast equilibration or non-fractionating exchange with the surrounding medium (Horner et al., 2013). In contrast, Cd-tolerant hyperaccumulator plants, grown in hydroponics, were enriched in light Cd isotopes relative to the nutrient solutions (Wei et al., 2016). Cadmium uptake could occur via metal transporters or ion channels that may impart covalent binding (Nelson and Cox, 2005). Hence, Cd uptake is expected to produce either an enrichment of light isotopes in plants, due to the Cd bonding with the transporter (Horner et al., 2011; Wasylenki et al., 2014; Zhang et al., 2016) or show no isotopic fractionation.

Overall, wheat and barley plants were observed to be isotopically heavier than soils, and this demonstrates that processes #1 and #2 were more important for the isotope fractionation between solid soils and plants than processes #3, #4, and #5. This finding is further confirmed with the positive correlation between the $\Delta^{114/110}Cd_{soil\text{-}plant}$ values (Figure 4) and the Cd stocks in plants. The higher the Cd stock in plants was, the isotopically lighter got the Cd in plants. This indicates that a pool-size effect after Cd desorption (#1) enhanced free Cd^{2+} concentrations at lower pH (#2) determined the apparent isotopic fractionation between soils and plants. In contrast, pool-size effects during Cd adsorption on root surfaces (#3), Cd diffusion (#4), or plant uptake of Cd (#5) would lead to isotopically heavier Cd in plants.

Cd Redistribution in Plants

Cd concentrations decrease from roots to straw and grains for both plants and all three sites (Figure S3) which is in line with previous observations (Page and Feller, 2015). The plants hence hindered transport of toxic Cd into the growing plant tissues. Such detoxification strategies are used by various organisms and might be similar between bacterial and human cells (Prévéral et al., 2009). In general, it is assumed that Cd is bound to phytochelatin or glutathione in the cytosol and excreted or sequestered afterwards, e.g. into vacuoles or cell walls (process #6, Figure 3, Clemens et al., 2002; Prévéral et al., 2009). This sequestration also causes isotopic effects with Cd isotope signatures that become heavier from roots to straw and grains. This observation is in line with results for wheat plants grown in a pot study on soils from the same locations (Wiggenhauser et al., 2016). Theoretical calculations showed

510 that sulfides are more enriched in light Cd isotopes than hydrated Cd (Yang et al. 2015). The
511 same isotope behavior was described for Hg which forms very strong Hg-S bonds, with the
512 lighter isotopes enriched in the bonding due to soft-acid soft-base interactions and lower
513 vibrational frequencies (Wiederhold, 2015). Consequently, the sequestered or excreted Cd (in
514 roots and straw, process #6, Figure 3) is isotopically lighter than the transported Cd (process
515 #7). Therefore, the new, emerging plant tissues show lower Cd concentrations and are
516 enriched in heavy Cd isotopes.

517 The Cd distribution and isotope fractionation in wheat can be described with a closed system
518 Rayleigh fractionation model (Figure 5, Wiederhold, 2015; Wiggenhauser et al., 2016). A pot
519 study thereby revealed different isotope fractionation factors for Cd retention in roots
520 compared to shoots, in roots and straw compared to grains, and in straw compared to grains
521 (grey lines in Figure 5, Wiggenhauser et al., 2016). The model assumes that the extent of the
522 isotope fractionation depends on the amount of Cd that is translocated from one plant part to
523 the next. To compare controlled conditions with field conditions, our new data for plants
524 grown at OE, WI and NE and the pot study results are both shown in Figure 5. Notably, the
525 Rayleigh fractionation lines that were fitted for the combined wheat data of both studies
526 (colored lines in Figure 5), show only marginal differences to the best fit lines for the pot-
527 study data only. Hence, the isotopic fractionation does not vary strongly between greenhouse
528 and field, different soil types and different wheat cultivars – for all three described
529 fractionation processes.

530 However, the apparent isotopic fractionation between roots-straw and grains (Figure 5b) as
531 well as between straw and grains (Figure 5c) differed between the two crops, although the
532 mass fractions of Cd transported into grains were in the same range for barley and wheat (11
533 to 44%, Figures 5b and 5c). Thus, the processes involved in grain filling might differ between
534 the wheat and barley cultivars chosen in this study. Isotope labeling studies showed that Cd is
535 directly transported from root to grain and remobilized from stem and leaves during grain
536 filling of wheat (Riesen and Feller, 2005; Yan et al., 2018; Harris and Taylor, 2001). The main
537 processes that determine remobilization and metal isotope fractionation might be adsorption
538 in the apoplastic space, complexation and compartmentalization in the cells, phloem mobility,
539 and membrane transport (Wiggenhauser et al. 2018). The distinct apparent isotope
540 fractionation suggests that the contribution of the processes that control remobilization

differed between wheat and barley. For instance, Cd retention in straw induced by complexation to thiol ligands is expected to cause a stronger retention of light isotopes compared to binding to carboxylates (apoplastic binding sites) if bond lengths not only control the direction but also the extent of isotope fractionation (see section “Cd in soil and soil solution”). However, more experimental data is needed to strengthen such isotope process tracing approaches for Cd. Furthermore, the contributions from remobilized Cd to grain Cd differs among wheat cultivars (Harris and Taylor, 2001) which suggests the differences found in this study could be also caused by the wheat and barley cultivars and not only by the different plants species. We only found one study that directly compared (net) remobilization patterns of wheat and barley (Maillard et al. 2015). It revealed that the net remobilization of most micronutrients is similar in wheat and barley when compared to e.g. maize, rape, and pisum sativum. To our knowledge, no such comparison exists for Cd. Together, our isotope analysis showed that processes that determine the Cd transport into the grains can differ between wheat and barley.

Conclusion

i) The isotope fractionation between soil and soil solution was not affected by any other property of the soil solid phase or soil solution. The constant isotopic fractionation between the sites suggests that Cd was primarily bound to organic matter and hydroxides as inner-sphere complex. The heavier isotope compositions in the soil solution compared to soil extracts indicate a pool-size effect controlling the Cd isotope composition of the Cd in soil solution.

ii) Desorption from soil, soil solution speciation, adsorption on root surfaces, diffusion, and plant uptake were identified as the responsible processes for the Cd isotope fractionation between soil, soil solution and plants. Thereby, the first two process dominated over the other processes. This valuable finding can be used in future research in which Cd stable isotopes are involved.

iii) Within plants, compartments with lower Cd concentrations were enriched in light isotopes, following a Rayleigh fractionation model. The fractionation and retention might be a consequence of Cd retention mechanisms, cereals use to prevent the transport of the toxic Cd to the grain. Barley cultivars were more efficient in Cd retention, showing lower Cd concentrations and stronger isotope fractionation, than wheat cultivars.

Supplementary Material

Section 1: Material and methods (detailed information). Figure S1: Experimental sites. Figure S2: Selected soil properties. Figure S3: Plant properties. Figure S4: Relationship between soil and plant Cd concentrations. Figure S5: Comparison of expected with measured Cd stocks in wheat and barley. Table S1: Soil solution properties. Table S2: Soil properties. Table S3: Standard reference materials.

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References

Allen, R.G., 1998. Crop evapotranspiration: Guidelines for computing crop water requirements, FAO irrigation and drainage paper. Rome.

Alloway, B.J., Steinnes, E., 1999. Anthropogenic Additions of Cadmium to Soils, in: Cadmium in Soils and Plants. Springer Netherlands, Dordrecht, pp. 97–123. https://doi.org/10.1007/978-94-011-4473-5_5

Awad, F., Römheld, V., 2000. Mobilization of heavy metals from contaminated calcareous soils by plant born, microbial and synthetic chelators and their uptake by wheat plants. J. Plant Nutr. 23, 1847–1855. https://doi.org/10.1080/01904160009382147

Balistrieri, L.S., Borrok, D.M., Wanty, R.B., Ridley, W.I., 2008. Fractionation of Cu and Zn isotopes during adsorption onto amorphous Fe(III) oxyhydroxide: Experimental mixing of acid rock drainage and ambient river water. Geochim. Cosmochim. Acta 72, 311–328. https://doi.org/10.1016/j.gca.2007.11.013

Bigalke, M., Weyer, S., Wilcke, W., 2010. Copper isotope fractionation during complexation with insolubilized humic acid. Environ. Sci. Technol. 44, 5496–5502. https://doi.org/10.1021/es1017653

Blume, H.-P., Brümmer, G.W., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., Thiele-Bruhn, S., Welp, G., Wilke, B.-M., 2010. Scheffer / Schachtschabel Lehrbuch der Bodenkunde, 16. Auflag. ed. Spektrum Akademischer Verlag, Heidelberg. https://doi.org/10.1007/978-3-8274-2251-4

Bochatay, L., Persson, P., Sjöberg, S., 2000. Metal Ion Coordination at the Water-Manganite (gamma-MnOOH) Interface. J. Colloid Interface Sci. 229, 584–592. https://doi.org/10.1006/jcis.2000.7013

Bruemmer, G.W., Gerth, J., Herms, U., 1986. Heavy metal species, mobility and availability in soils. Zeitschrift für Pflanzenernährung und Bodenkd. 149, 382–398. https://doi.org/10.1002/jpln.19861490404

Bryan, A.L., Dong, S., Wilkes, E.B., Wasylenki, L.E., 2015. Zinc isotope fractionation during adsorption onto Mn oxyhydroxide at low and high ionic strength. Geochim. Cosmochim.

619 Acta 157, 182–197. https://doi.org/10.1016/j.gca.2015.01.026

620 Buekers, J., Degryse, F., Maes, A., Smolders, E., 2008. Modelling the effects of ageing on Cd,
621 Zn, Ni and Cu solubility in soils using an assemblage model. Eur. J. Soil Sci. 59, 1160–
622 1170. https://doi.org/10.1111/j.1365-2389.2008.01053.x

623 Cancès, B., Ponthieu, M., Castrec-Rouelle, M., Aubry, E., Benedetti, M.F., 2003. Metal ions
624 speciation in a soil and its solution: experimental data and model results. Geoderma 113,
625 341–355. https://doi.org/10.1016/S0016-7061(02)00369-5

626 Chen, F., Wu, F., Dong, J., Vincze, E., Zhang, G., Wang, F., Huang, Y., Wei, K., 2007. Cadmium
627 translocation and accumulation in developing barley grains. Planta 227, 223–232.
628 https://doi.org/10.1007/s00425-007-0610-3

629 Christensen, T.H., 1984. Cadmium soil sorption at low concentrations: I. Effect of time,
630 cadmium load, pH, and calcium. Water. Air. Soil Pollut. 21, 105–114.
631 https://doi.org/10.1007/BF00163616

632 Clemens, S., Ma, J.F., 2016. Toxic Heavy Metal and Metalloid Accumulation in Crop Plants and
633 Foods. Annu. Rev. Plant Biol. 67, 489–512. https://doi.org/10.1146/annurev-arplant-
634 043015-112301

635 Clemens, S., Palmgren, M.G., Krämer, U., 2002. A long way ahead: Understanding and
636 engineering plant metal accumulation. Trends Plant Sci. 7, 309–315.
637 https://doi.org/10.1016/S1360-1385(02)02295-1

638 Collins, R.N., Merrington, G., McLaughlin, M.J., Morel, J.L., 2003. Organic ligand and pH effects
639 on isotopically exchangeable cadmium in polluted soils. Soil Sci. Soc. Am. J. 67, 112–121.
640 https://doi.org/10.2136/sssaj2003.1120

641 Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-
642 nutrient environments. Plant Soil 245, 35–47.

643 de Groot, A.C., Peijnenburg, W.J.G.M., van den Hoop, M.A.G.T., Ritsema, R., van Venn, R.P.M.,
644 1998. Heavy Metals in Dutch field soils: an experimental and theoretical study on
645 equilibrium partitioning. Rijksinstituut voor Volksgezondheid en Milieu RIVM.

646 Degryse, F., Broos, K., Smolders, E., Merckx, R., 2003. Soil solution concentration of Cd and Zn
647 can be predicted with a CaCl2 soil extract. Eur. J. Soil Sci. 54, 149–157.
648 https://doi.org/10.1046/j.1365-2389.2003.00503.x

649 Dong, S., Wasylenki, L.E., 2016. Zinc isotope fractionation during adsorption to calcite at high
650 and low ionic strength. Chem. Geol. 447, 70–78.
651 https://doi.org/10.1016/j.chemgeo.2016.10.031

652 Dorcak, V., Krezel, A., 2003. Correlation of acid-base chemistry of phytochelatin PC2 with its
653 coordination properties towards the toxic metal ion Cd(II). Dalton Trans. 2253.
654 https://doi.org/10.1039/b301357j

655 Farrah, H., Hatton, D., Pickering, W.F., 1980. The affinity of metal ions for clay surfaces. Chem.
656 Geol. 28, 55–68. https://doi.org/10.1016/0009-2541(80)90035-2

657 Fulda, B., Voegelin, A., Kretzschmar, R., 2013. Redox-controlled changes in cadmium solubility
658 and solid-phase speciation in a paddy soil as affected by reducible sulfate and copper.
659 Environ. Sci. Technol. 47, 12775–12783. https://doi.org/10.1021/es401997d

660 Fulton, J., Hoffmann, M., Darab, J., Palmer, B., Stern, E., 2000. Copper(I) and Copper(II)
661 Coordination Structure under Hydrothermal Conditions at 325 °C: An X-ray Absorption
662 Fine Structure and Molecular Dynamics Study. J. Phys. Chem. A 104, 11651–11663.
663 https://doi.org/10.1021/jp001949a

664 Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., Groneberg,
665 D.A., 2006. The toxicity of cadmium and resulting hazards for human health. J. Occup.
666 Med. Toxicol. 1, 22. https://doi.org/10.1186/1745-6673-1-22

667 Gray, C.W., McLaren, R.G., Roberts, A.H.C., Condron, L.M., 1999. Cadmium phytoavailability in
668 some New Zealand soils. Aust. J. Soil Res. 37, 461. https://doi.org/10.1071/S98070

669 Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison
670 with annual plants: the importance of root exudation and its impact on microbial activity
671 and nutrient availability. Appl. Soil Ecol. 5, 29–56. https://doi.org/10.1016/S0929-
672 1393(96)00126-6

673 Groenenberg, J.E., Koopmans, G.F., Comans, R.N.J., 2010. Uncertainty analysis of the nonideal

competitive adsorption - Donnan model: Effects of dissolved organic matter variability on predicted metal speciation in soil solution. Environ. Sci. Technol. 44, 1340–1346. https://doi.org/10.1021/es902615w

Harris, N.S., Taylor, G.J., 2001. Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation. J. Exp. Bot. 52, 1473–1481. https://doi.org/10.1093/jexbot/52.360.1473

Herms, U., Brümmer, G., 1984. Einflußgrößen der Schwermetallöslichkeit und -bindung in Böden. Zeitschrift für Pflanzenernährung und Bodenkd. 147, 400–424. https://doi.org/10.1002/jpln.19841470313

Hindshaw, R.S., Bourdon, B., Pogge von Strandmann, P.A.E., Vigier, N., Burton, K.W., 2013. The stable calcium isotopic composition of rivers draining basaltic catchments in Iceland. Earth Planet. Sci. Lett. 374, 173–184. https://doi.org/10.1016/j.epsl.2013.05.038

Horner, T.J., Lee, R.B.Y., Henderson, G.M., Rickaby, R.E.M., 2013. Nonspecific uptake and homeostasis drive the oceanic cadmium cycle. Proc. Natl. Acad. Sci. 110, 2500–2505. https://doi.org/10.1073/pnas.1213857110

Horner, T.J., Rickaby, R.E.M., Henderson, G.M., 2011. Isotopic fractionation of cadmium into calcite. Earth Planet. Sci. Lett. 312, 243–253. https://doi.org/10.1016/j.epsl.2011.10.004

Imseng, M., Wiggenhauser, M., Keller, A., Müller, M., Rehkämper, M., Murphy, K., Kreissig, K., Frossard, E., Wilcke, W., Bigalke, M., 2018. Fate of Cd in agricultural soils: A stable isotope approach to anthropogenic impact, soil formation and soil-plant cycling. Environ. Sci. Technol. 52, acs.est.7b05439. https://doi.org/10.1021/acs.est.7b05439

Isaure, M.P., Huguet, S., Meyer, C.L., Castillo-Michel, H., Testemale, D., Vantelon, D., Saumitou-Laprade, P., Verbruggen, N., Sarret, G., 2015. Evidence of various mechanisms of Cd sequestration in the hyperaccumulator Arabidopsis halleri, the non-accumulator Arabidopsis lyrata, and their progenies by combined synchrotron-based techniques. J. Exp. Bot. 66, 3201–3214. https://doi.org/10.1093/jxb/erv131

Jouvin, D., Louvat, P., Juillot, F., Maréchal, C.N., Benedetti, M.F., 2009. Zinc isotopic fractionation: Why organic matters. Environ. Sci. Technol. 43, 5747–5754.

https://doi.org/10.1021/es803012e

Jouvin, D., Weiss, D.J., Mason, T.F.M., Bravin, M.N., Louvat, P., Zhao, F., Ferec, F., Hinsinger, P., Benedetti, M.F., 2012. Stable isotopes of Cu and Zn in higher plants: Evidence for Cu reduction at the root surface and two conceptual models for isotopic fractionation processes. Environ. Sci. Technol. 46, 2652–2660. https://doi.org/10.1021/es202587m

Juillot, F., Maréchal, C., Ponthieu, M., Cacaly, S., Morin, G., Benedetti, M., Hazemann, J.L., Proux, O., Guyot, F., 2008. Zn isotopic fractionation caused by sorption on goethite and 2-Lines ferrihydrite. Geochim. Cosmochim. Acta 72, 4886–4900. https://doi.org/10.1016/j.gca.2008.07.007

Karlsson, T., Persson, P., Skyllberg, U., 2005. Extended X-ray absorption fine structure spectroscopy evidence for the complexation of cadmium by reduced sulfur groups in natural organic matter. Environ. Sci. Technol. 39, 3048–3055. https://doi.org/10.1021/es048585a

Keller, A., Rossier, N., Desaules, A., 2005. Schwermetallbilanzen von Landwirtschaftsparzellen der nationalen Bodenbeobachtung: NABO - Nationales Bodenbeachtungsnetz der Schweiz, Schriftenreihe der FAL. FAL, Zürich.

Khan, M.A., Castro-Guerrero, N., Mendoza-Cozatl, D.G., 2014. Moving toward a precise nutrition: preferential loading of seeds with essential nutrients over non-essential toxic elements. Front. Plant Sci. 5, 51. https://doi.org/10.3389/fpls.2014.00051

Kubo, K., Kobayashi, H., Fujita, M., Ota, T., Minamiyama, Y., Watanabe, Y., Nakajima, T., Shinano, T., 2016. Varietal differences in the absorption and partitioning of cadmium in common wheat (Triticum aestivum L.). Environ. Exp. Bot. 124, 79–88. https://doi.org/10.1016/j.envexpbot.2015.12.007

Kusonwiriyawong, C., Bigalke, M., Abgottspon, F., Lazarov, M., Schuth, S., Weyer, S., Wilcke, W., 2017. Isotopic variation of dissolved and colloidal iron and copper in a carbonatic floodplain soil after experimental flooding. Chem. Geol. 459, 13–23. https://doi.org/10.1016/j.chemgeo.2017.03.033

Kuzmin, A., Obst, S., Purans, J., 1997. X-ray absorption spectroscopy and molecular dynamics

730 studies of Zn+2 hydration in aqueous solutions. J. Phys. Condens. Matter 9, 10065–
731 10078. https://doi.org/10.1088/0953-8984/9/46/004

732 Leff, B., Ramankutty, N., Foley, J.A., 2004. Geographic distribution of major crops across the
733 world. Global Biogeochem. Cycles 18, n/a-n/a. https://doi.org/10.1029/2003GB002108

734 Loganathan, P., Hedley, M.J., 1997. Downward movement of cadmium and phosphorus from
735 phosphatic fertilisers in a pasture soil in New Zealand. Environ. Pollut. 95, 319–324.
736 https://doi.org/10.1016/S0269-7491(96)00142-X

737 Maillard, A., Diquelou, S., Billard, V., Laine, P., Garnica, M., Prudent, M., Garcia-Mina, J.-M.,
738 Yvin, J.-C., Ourry, A., 2015. Leaf mineral nutrient remobilization during leaf senescence
739 and modulation by nutrient deficiency. Front. Plant Sci. 6, 317.
740 https://doi.org/10.3389/fpls.2015.00317

741 Malferrari, D., Brigatti, M.F., Laurora, A., Pini, S., Medici, L., 2007. Sorption kinetics and
742 chemical forms of Cd(II) sorbed by thiol-functionalized 2:1 clay minerals. J. Hazard.
743 Mater. 143, 73–81. https://doi.org/10.1016/j.jhazmat.2006.08.069

744 Maret, W., Moulis, J.-M., 2013. The Bioinorganic Chemistry of Cadmium in the Context of Its
745 Toxicity, in: Metal Ions in Life Sciences. pp. 1–29. https://doi.org/10.1007/978-94-007-
746 5179-8_1

747 McBride, M.B., 2002. CADMIUM UPTAKE BY CROPS ESTIMATED FROM SOIL TOTAL Cd AND
748 pH. Soil Sci. 167, 62–67. https://doi.org/10.1097/00010694-200201000-00006

749 Mendoza-Cózatl, D.G., Jobe, T.O., Hauser, F., Schroeder, J.I., 2011. Long-distance transport,
750 vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium
751 and arsenic. Curr. Opin. Plant Biol. https://doi.org/10.1016/j.pbi.2011.07.004

752 MeteoSwiss, 2015. Federal Office of Meteorology and Climatology MeteoSwiss [WWW
753 Document]. URL www.meteoswiss.admin.ch

754 Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for
755 plants: a review. Environ. Chem. Lett. 8, 199–216. https://doi.org/10.1007/s10311-010-
756 0297-8

Naidu, R., Harter, R.D., 1998. Effect of Different Organic Ligands on Cadmium Sorption by and Extractability from Soils. Soil Sci. Soc. Am. J. 62, 644–650. https://doi.org/10.2136/sssaj1998.03615995006200030014x

Nelson, D.L., Cox, M.M., 2005. Lehninger Principles of Biochemistry.

Nelson, J., Wasylenki, L., Bargar, J.R., Brown, G.E., Maher, K., 2017. Effects of surface structural disorder and surface coverage on isotopic fractionation during Zn(II) adsorption onto quartz and amorphous silica surfaces. Geochim. Cosmochim. Acta 215, 354–376. https://doi.org/10.1016/j.gca.2017.08.003

Nolan, A.L., McLaughlin, M.J., Mason, S.D., 2003. Chemical speciation of Zn, Cd, Cu, and Pb in pore waters of agricultural and contaminated soils using donnan dialysis. Environ. Sci. Technol. 37, 90–98. https://doi.org/10.1021/es025966k

Nordberg, G.F., 2009. Historical perspectives on cadmium toxicology. Toxicol. Appl. Pharmacol. https://doi.org/10.1016/j.taap.2009.03.015

Ockert, C., Gussone, N., Kaufhold, S., Teichert, B.M.A., 2013. Isotope fractionation during Ca exchange on clay minerals in a marine environment. Geochim. Cosmochim. Acta 112, 374–388. https://doi.org/10.1016/j.gca.2012.09.041

Ohtaki, H., Johansson, G., 1981. X-Ray Diffraction Studies on the Structures of Cadmium Iodide Complexes in Water and in DMSO Solutions. Pure Appl. Chem. 53, 1357–1364. https://doi.org/10.1351/pac198153071357

Page, V., Feller, U., 2015. Heavy Metals in Crop Plants: Transport and Redistribution Processes on the Whole Plant Level. Agronomy 5, 447–463. https://doi.org/10.3390/agronomy5030447

Peacock, C.L., Sherman, D.M., 2004. Copper(II) sorption onto goethite, hematite and lepidocrocite: A surface complexation model based on ab initio molecular geometries and EXAFS spectroscopy. Geochim. Cosmochim. Acta 68, 2623–2637. https://doi.org/10.1016/j.gca.2003.11.030

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., Forestier, C., 2002. Heavy metal toxicity: Cadmium permeates through calcium channels and disturbs the plant water status. Plant

785 J. 32, 539–548. https://doi.org/10.1046/j.1365-313X.2002.01442.x

786 Pokrovsky, O.S., Viers, J., Emnova, E.E., Kompantseva, E.I., Freydier, R., 2008. Copper isotope
787 fractionation during its interaction with soil and aquatic microorganisms and metal
788 oxy(hydr)oxides: Possible structural control. Geochim. Cosmochim. Acta 72, 1742–1757.
789 https://doi.org/10.1016/j.gca.2008.01.018

790 Prévéral, S., Gayet, L., Moldes, C., Hoffmann, J., Mounicou, S., Gruet, A., Reynaud, F., Lobinski,
791 R., Verbavatz, J.-M., Vavasseur, A., Forestier, C., 2009. A common highly conserved
792 cadmium detoxification mechanism from bacteria to humans: heavy metal tolerance
793 conferred by the ATP-binding cassette (ABC) transporter SpHMT1 requires glutathione
794 but not metal-chelating phytochelatin peptides. J. Biol. Chem. 284, 4936–43.
795 https://doi.org/10.1074/jbc.M808130200

796 Pribyl, D.W., 2010. A critical review of the conventional SOC to SOM conversion factor.
797 Geoderma 156, 75–83. https://doi.org/10.1016/j.geoderma.2010.02.003

798 Puschenreiter, M., Gruber, B., Wenzel, W.W., Schindlegger, Y., Hann, S., Spangl, B.,
799 Schenkeveld, W.D.C., Kraemer, S.M., Oburger, E., 2017. Phytosiderophore-induced
800 mobilization and uptake of Cd, Cu, Fe, Ni, Pb and Zn by wheat plants grown on metal-
801 enriched soils. Environ. Exp. Bot. 138, 67–76.
802 https://doi.org/10.1016/j.envexpbot.2017.03.011

803 Ren, Z.-L., Tella, M., Bravin, M.N., Comans, R.N.J., Dai, J., Garnier, J.-M., Sivry, Y., Doelsch, E.,
804 Straathof, A., Benedetti, M.F., 2015. Effect of dissolved organic matter composition on
805 metal speciation in soil solutions. Chem. Geol. 398, 61–69.
806 https://doi.org/10.1016/j.chemgeo.2015.01.020

807 Riesen, O., Feller, U., 2005. Redistribution of nickel, cobalt, manganese, zinc, and cadmium via
808 the phloem in young and maturing wheat. J. Plant Nutr. 28, 421–430.
809 https://doi.org/10.1081/PLN-200049153

810 Rodushkin, I., Stenberg, A., Andrén, H., Malinovsky, D., Baxter, D.C., 2004. Isotopic
811 Fractionation during Diffusion of Transition Metal Ions in Solution. Anal. Chem. 76,
812 2148–2151. https://doi.org/10.1021/ac035296g

Ryan, B.M., Kirby, J.K., Degryse, F., Scheiderich, K., McLaughlin, M.J., 2014. Copper isotope fractionation during equilibration with natural and synthetic ligands. Environ. Sci. Technol. 48, 8620–8626. https://doi.org/10.1021/es500764x

Sajidu, S.M.I., Persson, I., Masamba, W.R.L., Henry, E.M.T., 2008. Mechanisms of heavy metal sorption on alkaline clays from Tundulu in Malawi as determined by EXAFS. J. Hazard. Mater. 158, 401–409. https://doi.org/10.1016/j.jhazmat.2008.01.087

Sattelmacher, B., 2001. The apoplast and its significance for plant mineral nutrition. New Phytol. 149, 167–192. https://doi.org/10.1046/j.1469-8137.2001.00034.x

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden, and organic matter. Environ. Sci. Technol. 34, 1125–1131. https://doi.org/10.1021/es9907764

Schweizer, S.A., Seitz, B., van der Heijden, M.G.A., Schulin, R., Tandy, S., 2018. Impact of organic and conventional farming systems on wheat grain uptake and soil bioavailability of zinc and cadmium. Sci. Total Environ. 639, 608–616. https://doi.org/10.1016/j.scitotenv.2018.05.187

Smolders, E., Mertens, J., 2013. Cadmium: In: Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability, in: Springer.

Song, W.Y., Mendoza-Cózatl, D.G., Lee, Y., Schroeder, J.I., Ahn, S.N., Lee, H.S., Wicker, T., Martinoia, E., 2014. Phytochelatin-metal(loid) transport into vacuoles shows different substrate preferences in barley and Arabidopsis. Plant, Cell Environ. 37, 1192–1201. https://doi.org/10.1111/pce.12227

Treeby, M., Marschner, H., Römheld, V., 1989. Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial, and synthetic metal chelators. Plant Soil 114, 217–226. https://doi.org/10.1007/BF02220801

Uraguchi, S., Kiyono, M., Sakamoto, T., Watanabe, I., Kuno, K., 2009. Contributions of apoplasmic cadmium accumulation, antioxidative enzymes and induction of phytochelatins in cadmium tolerance of the cadmium-accumulating cultivar of black oat (Avena strigosa Schreb.). Planta 230, 267–276. https://doi.org/10.1007/s00425-009-

841 0939-x

842 Vasconcelos, I.F., Haack, E.A., Maurice, P.A., Bunker, B.A., 2008. EXAFS analysis of cadmium(II)
843 adsorption to kaolinite. Chem. Geol. 249, 237–249.
844 https://doi.org/10.1016/j.chemgeo.2008.01.001

845 Wasylenki, L.E., Swihart, J.W., Romaniello, S.J., 2014. Cadmium isotope fractionation during
846 adsorption to Mn oxyhydroxide at low and high ionic strength. Geochim. Cosmochim.
847 Acta 140, 212–226. https://doi.org/10.1016/j.gca.2014.05.007

848 Waychunas, G.A., Fuller, C.C., Davis, J.A., 2002. Surface complexation and precipitate
849 geometery for aqueous Zn(II) sorption on ferrihydrite I: X-ray absorption extended fine
850 structure spectroscopy analysis. Geochim. Cosmochim. Acta 66, 1119–1137.

851 Wei, R., Guo, Q., Wen, H., Liu, C., Yang, J., Peters, M., Hu, J., Zhu, G., Zhang, H., Tian, L., Han,
852 X., Ma, J., Zhu, C., Wan, Y., 2016. Fractionation of Stable Cadmium Isotopes in the
853 Cadmium Tolerant Ricinus communis and Hyperaccumulator Solanum nigrum. Sci. Rep.
854 6, 24309. https://doi.org/10.1038/srep24309

855 Welch, R.M., 1995. Micronutrient Nutrition of Plants. CRC. Crit. Rev. Plant Sci. 14, 49–49.
856 https://doi.org/10.1080/713608066

857 Wiederhold, J.G., 2015. Metal Stable Isotope Signatures as Tracers in Environmental
858 Geochemistry. Environ. Sci. Technol. 49, 2606–2624. https://doi.org/10.1021/es504683e

859 Wiggenhauser, M., Bigalke, M., Imseng, M., Müller, M., Keller, A., Murphy, K., Kreissig, K.,
860 Rehkämper, M., Wilcke, W., Frossard, E., 2016. Cadmium isotope fractionation in soil-
861 wheat systems. Environ. Sci. Technol. 50, 9223–9231.
862 https://doi.org/10.1021/acs.est.6b01568

863 Wiggenhauser, M., Bigalke, M., Imseng, M., Keller, A., Archer, C., Wilcke, W., Frossard, E.,
864 2018. Zinc isotope fractionation during grain filling of wheat and a comparison of zinc
865 and cadmium isotope ratios in identical soil-plant systems. New Phytol.
866 https://doi.org/10.1111/nph.15146

867 Wilcke, W., Krauss, M., Kobza, J., 2005. Concentrations and forms of heavy metals in Slovak
868 soils. J. Plant Nutr. Soil Sci. 168, 676–686. https://doi.org/10.1002/jpln.200521811

869 Yamaji, N., Ma, J.F., 2014. The node, a hub for mineral nutrient distribution in graminaceous
870 plants. Trends Plant Sci. 19, 556–563. https://doi.org/10.1016/j.tplants.2014.05.007

871 Yan, B.F., Nguyen, C., Pokrovsky, O.S., Candaudap, F., Coriou, C., Bussière, S., Robert, T.,
872 Cornu, J.Y., 2018. Contribution of remobilization to the loading of cadmium in durum
873 wheat grains: impact of post-anthesis nitrogen supply. Plant Soil 424, 591–606.
874 https://doi.org/10.1007/s11104-018-3560-6

875 Yang, J., Li, Y., Liu, S., Tian, H., Chen, C., Liu, J., Shi, Y., 2015. Theoretical calculations of Cd
876 isotope fractionation in hydrothermal fluids. Chem. Geol. 391, 74–82.
877 https://doi.org/10.1016/j.chemgeo.2014.10.029

878 Zhang, Y., Wen, H., Zhu, C., Fan, H., Luo, C., Liu, J., Cloquet, C., 2016. Cd isotope fractionation
879 during simulated- and natural weathering. Environ. Pollut. 216, 9–17.

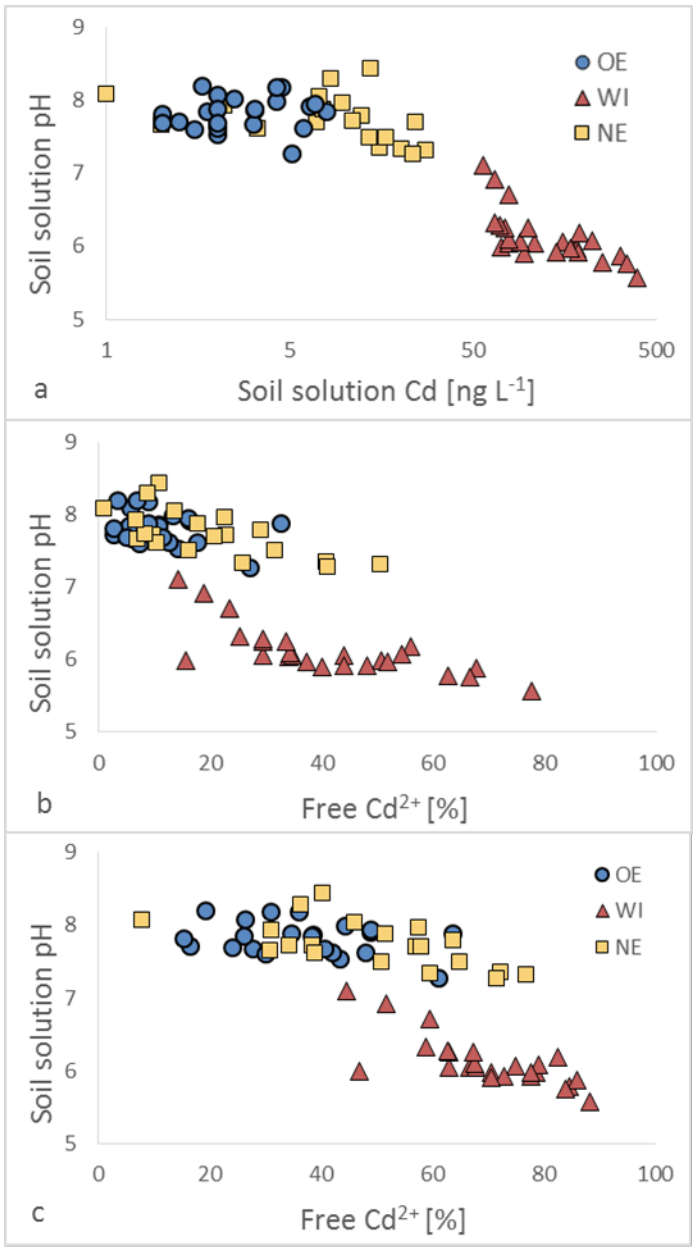
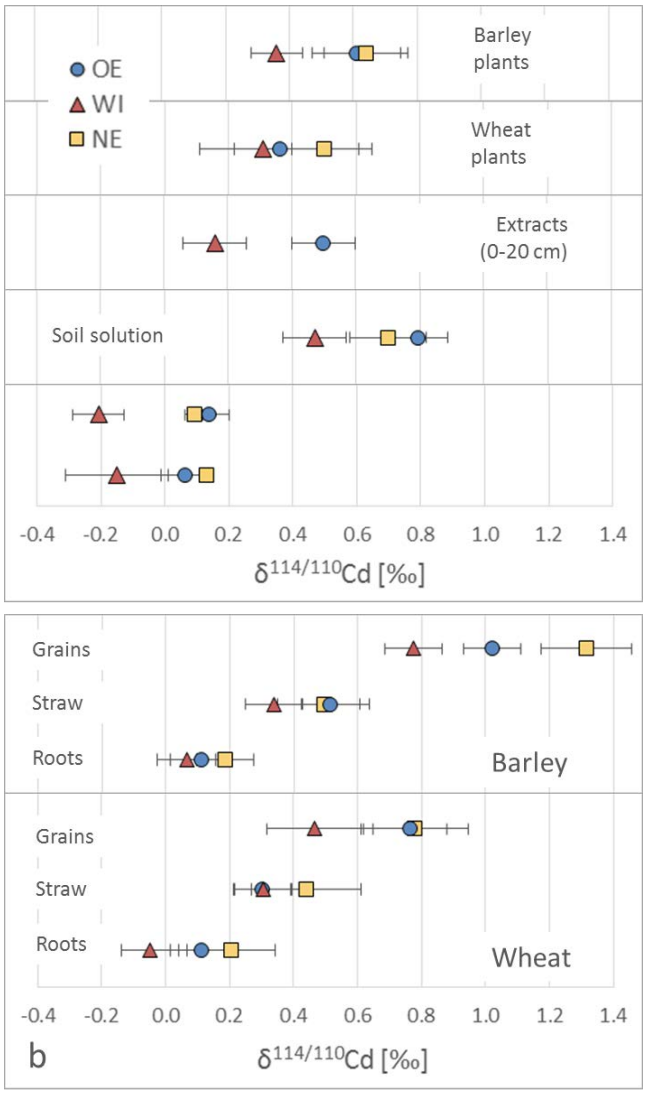


Figure 1. Relationship between measured soil solution pH and (a) measured soil solution Cd concentrations, (b) modelled fraction of free Cd^{2+} in soil solutions with the max-HS scenario, and (c) modelled fraction of free Cd^{2+} in soil solutions with the min-HS scenario. Max-HS and min-HS refer to the two model scenarios with maximum (60%) and minimum (20%) contributions of humic substances to total DOM concentrations. Each data point represents a biweekly measured or modelled value.



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889

890 Figure 2. Cd isotope compositions of (a) whole barley and wheat plants, 0.05 M $Ca(NO_3)_2$
891 extracts from soils (Wiggenhauser et al., 2016), soil solutions and soils (Imseng et al., 2018)
892 and (b) grains, straw, and roots of barley and wheat plants. Error bars represent 2 x standard
893 deviations of sample replicates where n>1 and measurement replicates where n=1. Minimal
894 2SD was the external reproducibility of 0.09‰. The isotope compositions of whole wheat and
895 barley plants were calculated according to Equation 3, with error propagation according to
896 Kusonwiriyawong et al. (2017).

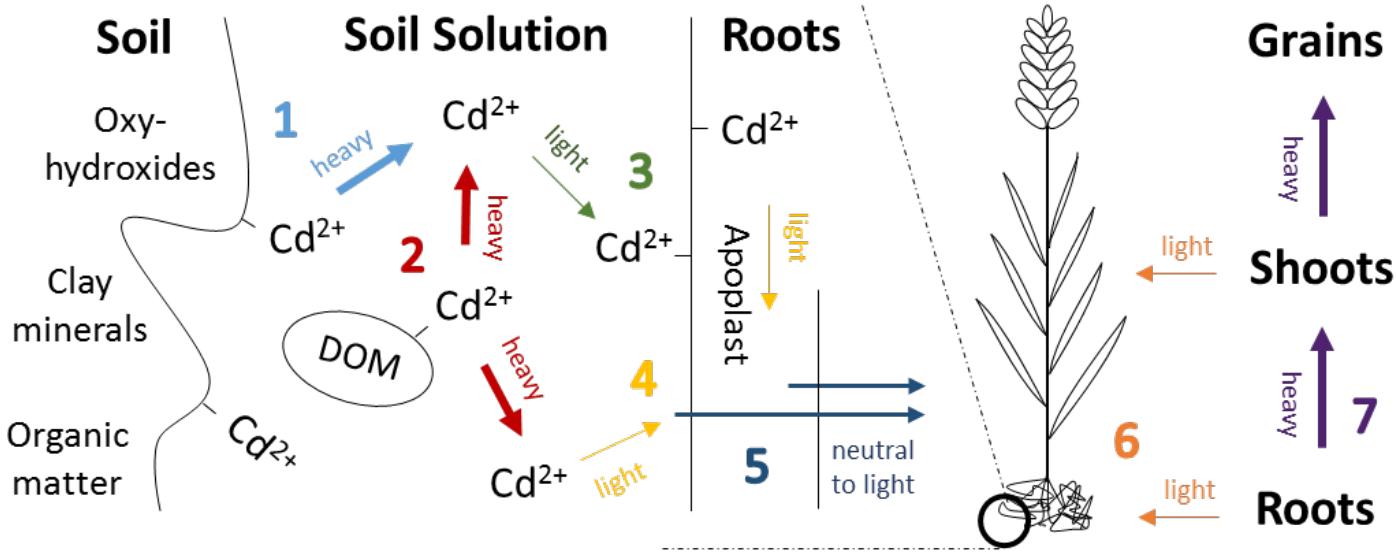
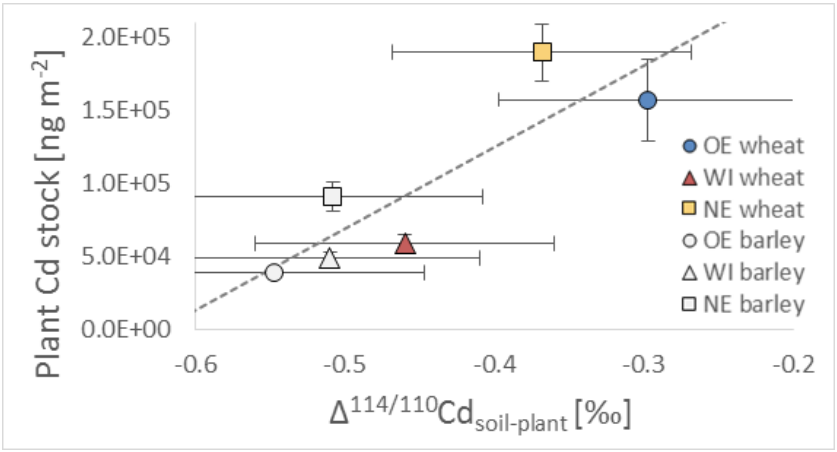


Figure 3. Suggested mechanisms for the Cd isotopic fractionation between soils, soil solutions, and plants and within plants. The thickness of the arrows illustrates the direction of the isotopic fractionation of the five processes in the rhizosphere (#1) Cd desorption from soils, (#2) Cd speciation in soil solutions, (#3) Cd adsorption on root surfaces, (#4) Cd diffusion, and (#5) plant uptake of Cd as well as the plant-internal redistribution processes Cd sequestration (#6) and Cd transport (#7).



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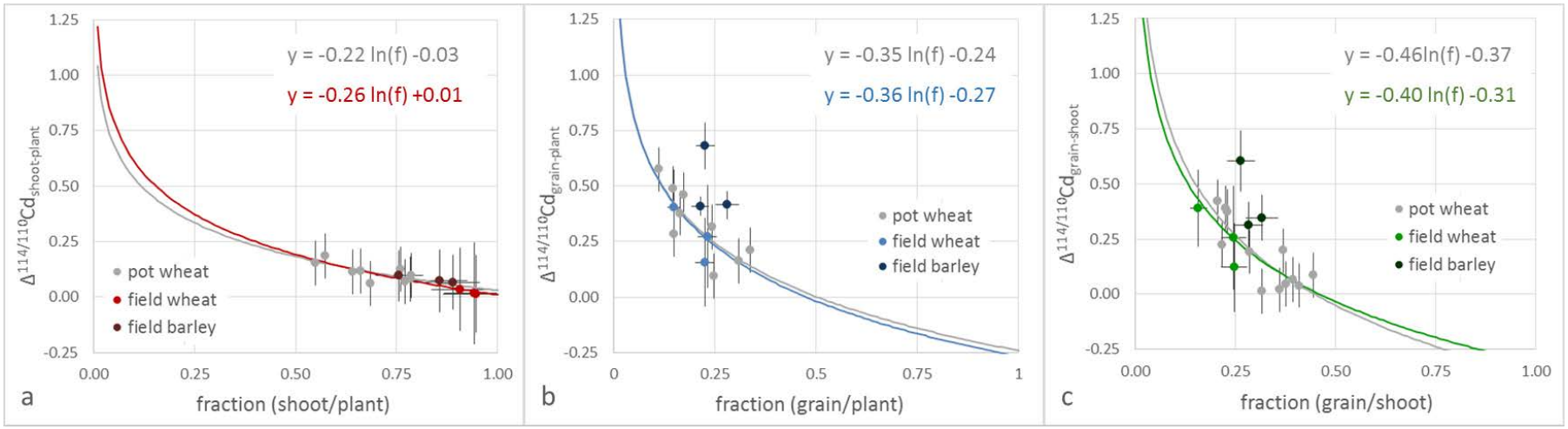
906 Figure 4. Relationship between the plant Cd stocks of wheat and barley and $\Delta^{114/110}Cd_{soil\text{-}plant}$
907 values. Error bars represent standard deviations of sample replicates where n>1 and
908 measurement replicates where n=1 and were calculated with error propagation according to
909 Kusonwiriyawong et al. (2017).



910

911 Figure 5. Rayleigh fractionation models for the field-grown wheat and barley plants of this study and wheat plants of a previous pot study. The best-
912 fit fractionation trends for the wheat of the pot experiment are shown as grey lines (Wiggenhauser et al., 2016), whereas the colored lines refer to
913 the best-fit fractionation trends for the wheat of pot and field experiments. (a) Cd retention in roots compared to shoots (straw and grains), (b) in
914 roots and straw compared to grains, and (c) in straw compared to grains. Error bars represent 1 x standard deviation for the mass fractions and 2 x
915 standard deviation for $\Delta^{114/110}$Cd values. Error propagation was calculated according to the equations from Kusonwiriyawong et al. (2017).